Porcine follicle-stimulating hormone treatment of gilts during an altrenogest-synchronized follicular phase: effects on follicle growth, hormone secretion, ovulation, and fertilization

H. D. Guthrie, V. G. Pursel and R. J. Wall

J Anim Sci 1997. 75:3246-3254.

Porcine Follicle-Stimulating Hormone Treatment of Gilts During an Altrenogest-Synchronized Follicular Phase: Effects on Follicle Growth, Hormone Secretion, Ovulation, and Fertilization[1,2]

H. D. Guthrie*,[3], V. G. Pursel†, and R. J. Wall†

*Germplasm and Gamete Physiology Laboratory and †Gene Evaluation and Mapping Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705

ABSTRACT: Porcine FSH (SUPER OV®), containing .03% LH activity, and equine chorionic gonadotropin (eCG) were administered during an altrenogest-synchronized follicular phase to determine their effects on follicle development, estrus, ovulation, and fertilization. Treatments were made by i.m. injection starting on d 1 (24 h after the last feeding of altrenogest): 1) saline, once, n = 14; 2) eCG (1,200 to 1,500 IU) once, n = 32; 3) FSH 14 (n = 2) or 21 (n = 6) NIH-FSH-S1 units/100 kg BW, divided among six injections at 12-h intervals (FSH14/21); 4) FSH, 28 NIH-FSH-S1 units/100 kg BW, divided among six injections at 12-h intervals, n = 12; and 5) FSH, 28 NIH-FSH-S1 units/100 kg BW and 100 IU hCG, two or six injections at 12-h intervals (FSH28+hCG), n = 13. Gilts were injected with 750 IU of hCG on d 5 to ensure ovulation. Twenty-eight eCG- and FSH-injected gilts (n = 6, 8, and 11 on treatments 3, 4, and 5, respectively) were bred and laparotomized on d 7 to recover ova and record ovulation rate. The mean number of ovulations and large (6- to 10-mm) follicles, respectively, on d 7 were as follows: saline (17, .7), eCG (43, .9), FSH14/21 (15, .6), FSH28 (12, 16), and FSH28+hCG (32, 21). Plasma FSH concentrations were at least threefold higher ($P < .05$) in gilts treated with FSH than in gilts not treated with FSH. The percentage in estrus was higher ($P < .05$) for saline- and eCG-treated gilts (100 and 87%, respectively) than for FSH-treated gilts (53%). Proportion of FSH28+hCG-treated gilts with fertilized ova (27%) was lower than for other groups (79 to 100%). In summary, the 3-d high dose FSH treatment (FSH28 and FSH28+hCG) during an altrenogest-synchronized follicular phase increased the number of potentially ovulatory follicles, but this potential benefit was not realized because many follicles failed to ovulate. The co-injection of low doses of hCG (FSH28+hCG) increased the ovulation rate and estradiol secretion but reduced ova recovery and fertilization rate compared with eCG and the other FSH treatments.

Key Words: FSH, Gilts, Estrous Cycle, Synchronization, Ovulation, Fertilization

J. Anim. Sci. 1997. 75:3246–3254

Introduction

Effective methods to induce superovulation and increase numbers of quality embryos have been elusive in swine. Combinations of pituitary gonadotropin preparations, equine chorionic gonadotropin (**eCG**), hCG, and GnRH have been used to induce growth of ovulatory follicles and ovulation in prepuberal gilts (Guthrie, 1977; Webel, 1978) during the follicular phase in the cycle (Hunter 1964), after weaning (Webel, 1978), after prostaglandin-induced luteolysis (Guthrie, 1979), and after estrus synchronization with agents such as methallibure and altrenogest (Webel, 1978).

Administration of eCG in swine increases the number of ova and early embryos (Day et al., 1967; Guthrie et al., 1974). However, the ovarian response to eCG is variable and treatment is associated with increased frequency of chromosomal abnormalities in ova (Koenig, 1987), degenerating embryos (Guthrie et al., 1974), embryonal mortality (Longenecker and Day, 1968), and no increase in litter size at term (Webel, 1978). Part of the variation associated with ovarian response in swine may be related to high amounts of LH biological activity present in most of the gonadotropin preparations. Preparations such as

[1]We thank Leah L. Schulman, Bruce S. Cooper, and Douglas W. Caldwell for their technical assistance.

[2]Mention of a trade name or proprietary product does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of others mentioned.

[3]To whom correspondence should be addressed: phone 301/504-9020, fax 301/504-5123.

Received March 25, 1997.
Accepted July 22, 1997.

Table 1. Treatment schedule

Group[a]	No. of gilts	Last fed altrenogest[b]	Promotion of follicle growth	Ovulation induction[c]
Saline	14	0800	Saline, at 1700 d 1	1300
eCG	32	1600	eCG, 1,200 or 1,500 IU, at 2100 on d 1	0700
FSH 14/21	8[d]	1600	FSH, 14 or 21 NIH FSH-S1 IU per 100 kg BW, divided over six injections at 0700 and 1900 on d 1, 2, and 3	0700
FSH 28	12	1600	FSH, 28 NIH FSH-S1 IU per 100 kg BW, divided over six injections at 0700 and 1900 on d 1, 2, and 3	0700
FSH 28+hCG	13	1600	FSH, 28 NIH FSH-S1 IU per 100 kg BW; divided over six injections at 0700 and 1900 on d 1, 2, and 3. Two injections of hCG 100 IU at 12-h intervals on d 1 or six injections at 12-h intervals on d 1, 2, and 3	0700

[a]eCG = equine chorionic gonadotropin; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin.
[b]Feed top-dressed with altrenogest once daily with last altrenogest fed on d 0.
[c]Gilts injected i.m. on d 5 with 750 IU of hCG.
[d]Two gilts received 14 and six gilts received 21 NIH-FSH-S1 units/100 kg BW, and the responses were averaged.

eCG and Shering Plough porcine FSH, commonly used to superovulate cattle, often contain as much LH as FSH receptor binding activity (Guthrie et al., 1990; Ausa, 1995). In cattle (Donaldson and Ward, 1986; Herrler et al., 1988), use of FSH preparations containing more than 5% LH relative to FSH activity decreased the number of healthy ova and live embryos.

In this study, we conducted an experiment with pigs during an altrenogest-synchronized follicular phase to determine the effects of an FSH preparation containing < 1% LH activity on ovarian response, plasma hormones, and fertilization after breeding. The rationale for conducting this study was that atretic follicles lost during selection of ovulatory follicles would be rescued by exogenous FSH, and the endogenous mechanisms of ovulatory follicle recruitment and maturation would act to increase the numbers of ovulatory follicles and numbers of fertilized ova after breeding.

Materials and Methods

Seventy-one crossbred gilts, weighing an average of 150 kg each, with previous estrous cycles of 19 to 21 d, were synchronized by feeding the progesterone agonist altrenogest (Regu-Mate®, Hoechst Roussel Pharmaceuticals, Somerville, NJ), at the rate of .17 mg/kg BW once daily for 5 d starting on d 11 to 15 of the estrous cycle. Porcine FSH (SUPER OV®, provided by Ausa International, Tyler, TX) and eCG (Diosynth, Chicago, IL) were administered i.m. to promote follicular growth. The FSH preparation contained a very low level of LH activity, .03% of FSH activity, as determined by radioligand assay relative to NIH-S1 FSH and LH standards (Ausa International, technical data sheet). Treatment was started on d 1; times of treatment and number of gilts assigned to each treatment group are given in Table 1. The gilts in group 1 receiving saline served as recipients for an embryo transfer study and were not bred. A boar was used for detection of estrus on d 1 through 6. Gilts in estrus on d 5 were bred to boars at the onset of estrus and 24 h later. Gilts not showing estrus by 1600 on d 5 were bred to boars or artificially inseminated at 0800 and 1600 on d 6. All gilts received one injection of 750 IU of hCG on d 5 at the time indicated in Table 1 to ensure ovulation. Blood was collected from each gilt on d 1 and 4, and at surgery on d 7 for plasma hormone assay. Sodium EDTA was used as anticoagulant, and plasma was stored at −20°C. On d 7, between 0900 and 1100, anesthesia was induced by administering the following per 100 kg of body weight: 400 mg ketamine HCl (Ketaset®, Aveco, Fort Dodge, IA); 200 mg xylazine (Rompun®, Haver Lockhart, Bayvet Division, Miles Laboratories, Shawnee, KS); 100 mg Telozol® (50 mg tiletamine HCl and 50 mg zolazepam HCl; Aveco); 4 mg butorphanol tartrate (Torbugesic®, Fort Dodge Laboratories, Fort Dodge, IA); and 6 mg atropine sulfate (Butler Company, Columbus, OH). One-fifth of the anesthetic dose was administered i.m., and the remaining dose was administered i.v. after 10 min. During anesthesia, each gilt's reproductive tract was exteriorized at midventral laparotomy to recover fertilized and unfertilized ova and count ovulations and unovulated small (1 to 2 mm), medium (3 to 6 mm), and large (> 6 mm) follicles. Fertilized and unfertilized ova were flushed from the oviducts in Beltsville Embryo Culture Medium (Pursel and Wall, 1996) and were held at 38°C while they were examined microscopically using formation of pronuclei as the criterion of fertilization.

Eight additional gilts were synchronized with altrenogest to obtain d-4 follicular fluid and more frequent blood samples for hormone assay. Two gilts each were assigned at random to one of four treatments indicated in Table 1: saline, eCG, FSH28, and FSH28+hCG. Blood for determination of plasma hormones was collected from gilts through a catheter placed nonsurgically into the vena cava on the last day of altrenogest administration (d 0). Blood samples were collected at the time of catheterization, at

8-h intervals until surgery on d 4, and at 15-min intervals from 0700 to 1100 and 1200 to 1600 on d 1 and 2 and from 0700 to 1100 on d 3. Sodium EDTA was used as an anticoagulant, and plasma was stored at –20°C. Each gilt's reproductive tract was exteriorized at midventral laparotomy as described above between 0830 and 1000 on d 4 to count follicles and aspirate a sample of follicular fluid from the 10 largest follicles from each gilt for steroid analysis.

The experimental protocols used in this research were approved by the Beltsville Area Institutional Animal Care and Use Committee.

Plasma Hormone Assay

Estradiol concentration was quantified in solvent extracts of .5 mL of plasma using a double-antibody [^{125}I]ligand radioimmunoassay kit (Pantex, Santa Monica, CA) as previously described (Guthrie et al., 1993). The average interassay CV for 230 aliquots of plasma replicated in two different assays was 9%, and the intraassay CV for a plasma sample repeated in four assays was 10%.

Concentrations of FSH and LH were quantified in duplicate aliquots of plasma with double-antibody radioimmunoassays (Guthrie et al., 1993). The standard was USDA-pFSH-I-1, the antibody was USDA-398-04p raised in a rabbit against USDA-FSH-PP1 β subunit, and the tracer was prepared with USDA-FSH-I-1. The average intraassay CV for 835 samples evaluated in duplicate in three assays was 12.9%. The average interassay CV for three samples repeated in each assay was 9.0%. For LH, the standard was USDA-pLH-B-1, the antibody was USDA-306-684p raised in a rabbit against pLH, and the tracer was prepared with USDA-LH-I-1. The average intraassay CV for 664 samples evaluated in duplicate in three assays was 14.9%, and the average interassay CV for three samples repeated in each assay was 11.4%.

Analysis of Follicular Fluid

Steroid concentrations were quantified without extraction using double-antibody [^{125}I]ligand radioimmunoassay kits (Estradiol and progesterone: Pantex; androstenedione: ICN, Costa Mesa, CA) (Guthrie et al., 1992). The minimum detectable amounts of estradiol, progesterone, and androstenedione were 1, 6, and 6 pg/tube, respectively. The average intraassay CV of one sample duplicated in each assay was 11, 6, and 4% for estradiol, progesterone, and androstenedione, respectively. The average interassay CV for 80 samples replicated in two assays was 13, 12, and 10% for estradiol, progesterone, and androstenedione, respectively.

Statistical Analysis

Data regression, analysis of variance, and chi-square analysis were performed on data using release 6.04 of the Statistical Analysis System package for personal computers (SAS, 1989). The frequency procedure for chi-square analysis was used to test for an association between gonadotropin treatment and proportion of gilts detected in estrus. The GLM procedure for analysis of variance was used to test plasma hormones for a two-way factorial effect of gonadotropin treatment and day after altrenogest. When the interaction component of the statistical model was significant (5% level), plasma hormone day means were compared for each treatment group, and d 4 means were compared among treatment groups. The statistical analysis of several ovarian and ova variables was a one-way analysis of variance testing the effect of gonadotropin treatment. When *F*-tests of analysis of variance were significant at the 5% level, multiple comparisons were made with the LSD method.

Results

The proportion of gilts detected in estrus differed among treatments. In saline- and eCG-treated gilts, 100 and 87%, respectively, were detected in estrus, compared to 53.2% in gilts in the three FSH-treated groups (χ^2 = 18, P < .001, n = 71). The proportion of gilts detected in estrus did not differ among the FSH treatment groups (χ^2 = 5, P = .234, n = 29).

Table 2 shows the mean ovarian responses on d 7 expressed on a per-gilt basis for each of the five treatment groups. The number of ovarian structures (ovulations, follicles > 1 mm, and cysts) was increased (P < .05) approximately 2 to 3.5 times by eCG, FSH28, and FSH28+hCG compared to saline and FSH14/21 treatment. Total number of ovarian structures was higher (P < .05) in FSH28+hCG-treated gilts than in gilts in the other treatment groups. The mean ovulation rate was highest in eCG-treated gilts, approximately 2.5 to 3 times higher (P < .05) than in saline-, FSH14/21-, and FSH28-treated gilts (Table 2). Ovulation rate in FSH28+hCG-treated gilts was higher (P < .05) than that in FSH14/21- and FSH28-treated gilts but did not differ from ovulation rate in eCG-treated gilts. The proportion of ovarian structures represented by ovulations was essentially 90% in gilts treated with saline, eCG, and FSH-14/21 (Table 2). The proportion of ovarian structures represented by ovulations was less (P < .05) in gilts treated with FSH-28 or FSH28+hCG than in gilts receiving other treatments due to an increased number of large follicles present on d 7. Ovarian cysts were present in six eCG-treated gilts and one FSH28+hCG gilt; however, incidence of ovarian cysts did not differ among groups (χ^2 = 7, P = .149, n = 71).

The conception rate (proportion of gilts with fertilized ova) was less (χ^2 = 15, P = .003, n = 53) in FSH28+hCG-treated gilts (27%) than in gilts in the other four treatment groups (Table 3). Of the 53

Table 2. Mean ovarian responses on day 7 to gonadotropin treatment

Treatment[a]	No. of gilts	Ovarian structures/gilt			
		Total no.	No. of CL	% CL	No. of large follicles
Saline	12	18.7[b]	17.5[bc]	93.8[b]	.7[b]
eCG	30	48.7[cd]	43.2[d]	89.9[b]	.9[b]
FSH14/21	8	16.5[b]	15.1[b]	92.6[b]	.6[b]
FSH28	10	37.7[c]	12.5[b]	38.3[c]	16.1[c]
FSH28+hCG	11	61.7[d]	31.9[cd]	57.8[d]	21.0[c]
SEM	—	2.4	2.1	2.2	1.3

[a]eCG = equine chorionic gonadotropin; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin.
[b,c,d]Means within a column lacking a common superscript letter differ by LSD test ($P < .05$).

inseminated gilts, 11 were not detected in estrus. The conception rate did not differ between gilts in estrus or not in estrus (74 and 64%, respectively, $\chi^2 = .4$, $P = .505$, n = 53). The mean total number of ova recovered per gilt was higher ($P < .05$) in eCG-treated than in FSH14/21-, FSH28-, or FSH28+hCG-treated gilts. The proportion of the ova per gilt represented by ovulations was lower ($P < .05$) in FSH28+hCG-treated (53%) than in eCG- (87%), FSH14/21- (80%), or FSH28- (93%) treated gilts. The mean number of fertilized ova recovered per gilt did not differ among all the gonadotropin-treated gilts (Table 3). Among gilts with fertilized ova, the proportion of the ova fertilized per gilt did not differ among treatment groups (Table 3).

Analysis of variance for plasma estradiol concentration showed that gonadotropin treatment ($P = .027$), day ($P < .001$), and the gonadotropin treatment × day interaction ($P < .006$) were significant sources of variation. Plasma estradiol increased between d 1 and 4 and then decreased between d 4 and 7 after the last administration of altrenogest (Table 4). The increase in plasma estradiol on d 4 was greater ($P \leq .05$) in eCG- and FSH28+hCG-treated gilts than in saline-, FSH14/21-, and FSH28-treated gilts. Plasma estradiol concentrations on d 4 did not differ among saline-, FSH14/21-, and FSH28-treated gilts.

Analysis of variance for plasma FSH showed that gonadotropin treatment ($P < .0001$), day ($P < .001$), and the gonadotropin treatment × day interaction ($P = .0001$) were significant sources of variation. Plasma FSH decreased between d 1 and 4 in saline-, eCG-, and FSH14/21-treated gilts, whereas concentrations did not differ among days for FSH28 and FSH28+hCG-treated gilts (Table 5). Plasma FSH concentration on d 4 in FSH14/21-treated gilts was greater than that of saline- and eCG-treated gilts, and less than that of FSH28-treated gilts.

Eight additional gilts were treated, two each, with saline, eCG, FSH28, or FSH28+hCG, and ovaries were examined on d 4 at laparotomy. Treatment with eCG, FSH28, or FSH28+hCG stimulated follicle growth ($P < .05$) compared to saline treatment (Table 6). The number of large follicles > 6 mm in diameter in eCG-, FSH28-, FSH28+hCG-treated gilts was 2.5 to 4 times greater ($P < .05$) than the number in saline-treated gilts. The number of large follicles in the FSH28- and FSH28+hCG-treated gilts was also greater ($P < .05$) than the number for eCG- treated gilts. The number of large follicles did not differ between FSH28- and FSH28+hCG-treated gilts.

Follicular steroid concentrations varied markedly, but they did not differ significantly among the

Table 3. Estrus, fertility, and ova recovery on day 7 in response to gonadotropin treatment

Treatment[a]	No. of gilts		Ova/gilt		
	Inseminated	With fertilized ova, %	Total no. recovered	No. fertilized	% Fertilized in gilts with fertilized ova
eCG	28	22 (79)	37.8[b]	17.9[b]	65.2[b]
FSH14/21	6	6 (100)	11.5[c]	10.0[b]	85.2[b]
FSH28	8	7 (88)	12.4[c]	8.4[b]	81.1[b]
FSH28+hCG	11	3 (27)	16.8[c]	8.4[b]	96.8[b]
SEM	—	—	2.7	2.0	4.7

[a]eCG = equine chorionic gonadotropin; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin.
[b,c]Means within a column lacking a common superscript letter differ by LSD test ($P < .05$).

Table 4. Mean plasma estradiol response to gonadotropin treatment

Gonadotropin treatment[a]	No. of gilts	Day after last administration of altrenogest 1	4	7
		pg/mL		
Saline	12	2.2^{B}	6.2^{bC}	3.0^{B}
eCG	24	2.9^{B}	15.3^{cC}	2.3^{B}
FSH14/21	8	2.4^{B}	6.2^{bC}	1.6^{B}
FSH28	8	3.2^{B}	7.7^{bC}	3.9^{B}
FSH28+hCG	6	2.5^{B}	12.4^{cC}	2.6^{B}
SEM	—	.1	1.3	.3

[a]eCG = equine chorionic gonadotropin; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin.

[b,c,d;B,C,D]Analysis of variance showed significant main effects and interaction between gonadotropin treatment and day. Preplanned comparisons were made for treatment effects among d 4 means and day effects for each gonadotropin mean. Means within a column lacking a common superscript lowercase letter and means in rows lacking a common superscript uppercase letter differ by LSD test ($P < .05$).

treatment groups on d 4, probably because of the small number of animals used (Table 6). However, estradiol concentration was low in the large follicles of one gilt treated with FSH28 and one gilt treated with FSH28+hCG, containing only 19 and 23 ng/mL of estradiol, respectively. Mean estradiol concentrations in follicular fluid of the other six gilts ranged from 217 to 400 ng/mL, well within the expected range.

Plasma FSH concentrations on d 4 for one gilt each receiving saline, eCG, FSH28, and FSH28+hCG are shown in Figure 1. Analysis of variance of the day means for each gilt showed that gonadotropin treatment ($P < .001$) and interaction of gonadotropin treatment × day ($P = .046$) were significant sources of variation in the data. Day was not a significant ($P = .549$) source of variation. Treatment with FSH28 or FSH28+hCG caused plasma FSH concentrations to increase compared to treatment with saline or eCG and remain elevated throughout the treatment period. In contrast, plasma FSH in saline- and eCG-treated gilts was lower on d 1 than in FSH28- and FSH28+hCG-treated gilts and decreased between d 1 and 3. Plasma FSH concentrations did not differ between FSH28- and FSH28+hCG-treated gilts. Plasma LH concentrations for the same gilts shown in Figure 1 are shown in Figure 2. Analysis of variance showed that day ($P = .003$) was a significant source of variation in the data, whereas gonadotropin treatment ($P = .87$) and interaction of gonadotropin treatment × day ($P = .724$) were not significant. The day means for each gilt did not differ among gonadotropin treatments and decreased between d 1 and 2. The frequency and amplitude of LH pulses was not analyzed statistically; however, the amplitude of pulsatile episodes seemed to decrease between d 1 and 2.

Table 5. Mean plasma FSH response to gonadotropin treatment

Gonadotropin treatment[a]	No. of gilts	Day after last administration of altrenogest 1	4	7
		ng/mL		
Saline	12	.48^{B}	.13^{Cb}	.42^{B}
eCG	24	.53^{B}	.10^{Cb}	.10^{C}
FSH14/21	8	.89^{B}	.41^{Cc}	.07^{D}
FSH28	8	.61^{B}	.81^{Bd}	.47^{B}
FSH28+hCG	3	.77^{B}	.68^{Bcd}	.16^{C}
SEM	—	.05	.04	.05

[a]eCG = equine chorionic gonadotropin; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin.

[b,c,d;B,C,D]Analysis of variance showed significant main effects and interaction between gonadotropin treatment and day. Preplanned comparisons were made for treatment effects among d 4 means and day effects for each gonadotropin mean. Means within a column lacking a common superscript lowercase letter and means in rows lacking a common superscript uppercase letter differ by LSD test ($P < .05$).

Plasma estradiol concentrations for the eight gilts are shown in Figure 3. Analysis of variance showed that gonadotropin treatment ($P = .047$) and day ($P = .007$) were significant sources of variation, but their interaction was not. Plasma estradiol increased between d 1 and 4 and increased to a higher concentration in gonadotropin-treated than in saline-treated gilts. Plasma estradiol concentration was markedly less in the two gilts (988 and 977) that had low estradiol concentration in their large follicles.

Discussion

Gilts undergoing an altrenogest-synchronized follicular phase show growth of large, estrogenic follicles by d 3 (d 0, last day of altrenogest administration) (Guthrie et al., 1993). These animals exhibit estrus and begin a preovulatory LH surge on the afternoon of d 5 or morning of d 6. Follicles excluded from the ovulatory cohort decrease in number and undergo atresia (Guthrie et al., 1993, 1994). The increased incidence of atresia among nonovulatory follicles is accompanied by decreased secretion of FSH that remains low until the preovulatory LH surge (Guthrie and Bolt, 1985; Guthrie et al., 1993). In this study, gilts receiving a total dose of 14 to 28 NIH-FSH-S1 units of FSH for 3 d had high circulating amounts of plasma FSH during treatment, and on d 2 and 3 plasma FSH concentrations were maintained at concentrations from three to nine times greater than for saline- or eCG-treated gilts.

The high concentrations of plasma FSH on d 1 to 3 in gilts treated with FSH28 and FSH28+hCG were consistent with the marked increase in number of large follicles on d 4 in these gilts compared to saline-

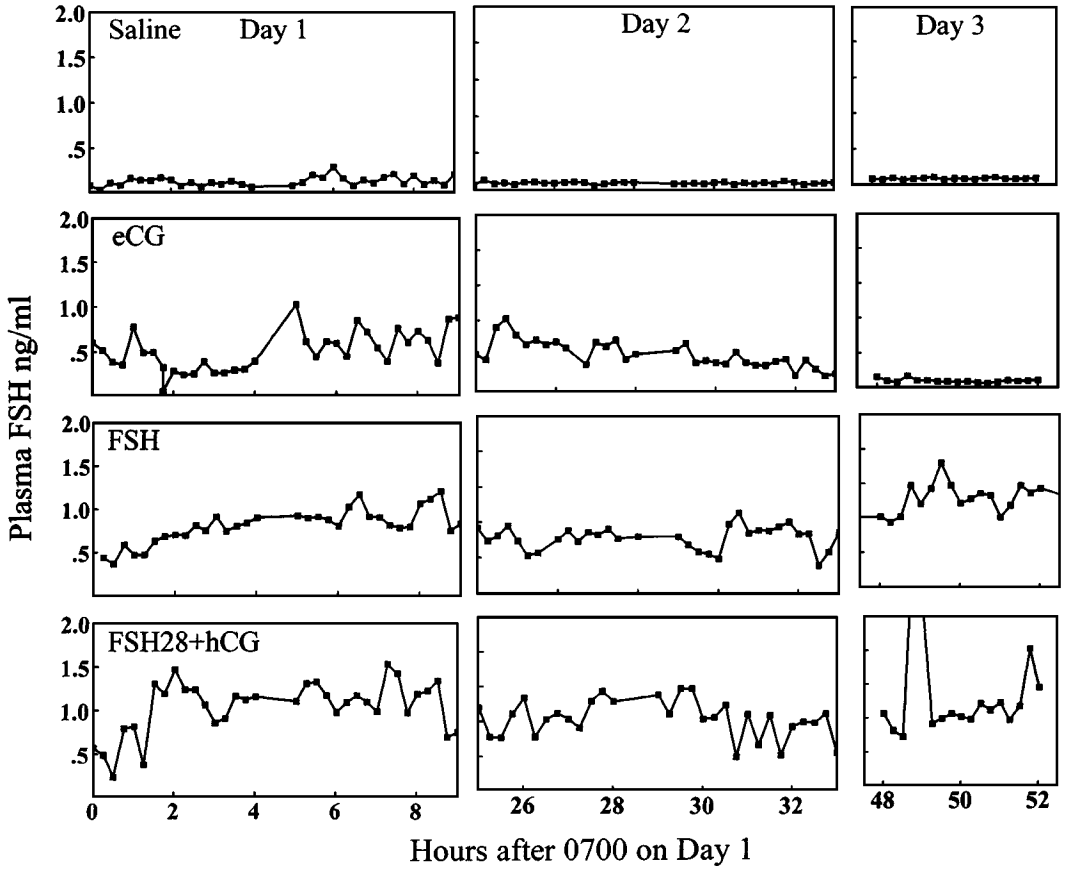

Figure 1. Follicle-stimulating hormone concentrations in blood collected at 15-min intervals from 0700 to 1100 and 1200 to 1600 on d 1 and 2 and from 0700 to 1100 on d 3 after the last feeding of altrenogest in four gilts. Gilts received the following treatments starting on d 1: one injection of saline at 2100, one injection of 1,200 IU of equine chorionic gonadotropin (eCG) at 2100, six injections of follicle-stimulating hormone (FSH) (total dose 28 NIH-S1 units/100 kg BW) at 12-h intervals starting at 0700 on d 1, and six injections of FSH (total dose 28 NIH-S1 units/100 kg BW) and 100 IU of human chorionic gonadotropin (hCG) at 12-h intervals starting at 0700 on d 1.

treated gilts. The most striking characteristic in FSH28- and FSH28+hCG-treated pigs was the discrepancy between the number of large follicles present on d 4 and the number of ovulations on d 7. A mean of 60 large follicles were present on d 4 in FSH28- and FSH28+hCG-treated gilts. However, the total number of ovulations and large follicles, respectively, present on d 7 were reduced to 12 and 16 (FSH28) and 32 and 21 (FSH28+hCG). All gilts receiving eCG or FSH were injected with an ovulating dose of hCG at 0700 on d 5. Based on results of earlier studies, ovulation should have been complete 42 h after the hCG

Table 6. Ovarian responses on day 4 to gonadotropin treatment

		No. of follicles		Follicular fluid hormones, ng/mL		
Treatment[a]	No. of gilts	≥ 2 mm	> 6 mm	Estradiol	Androstenedione	Progesterone
Saline	2	27.0[b]	15.5[b]	310[b]	95[b]	80[b]
eCG	2	58.5[bc]	43.0[c]	287[b]	97[b]	709[b]
FSH	2	73.5[c]	60.5[d]	190[b]	49[b]	201[b]
FSH+eCG	2	86.0[c]	63.0[d]	120[b]	222[b]	657[b]
SEM	—	5.0	2.0	54	28	99

[a]eCG = equine chorionic gonadotropin; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin.
[b,c,d]Means within a column lacking a common superscript letter differ by LSD test ($P < .05$).

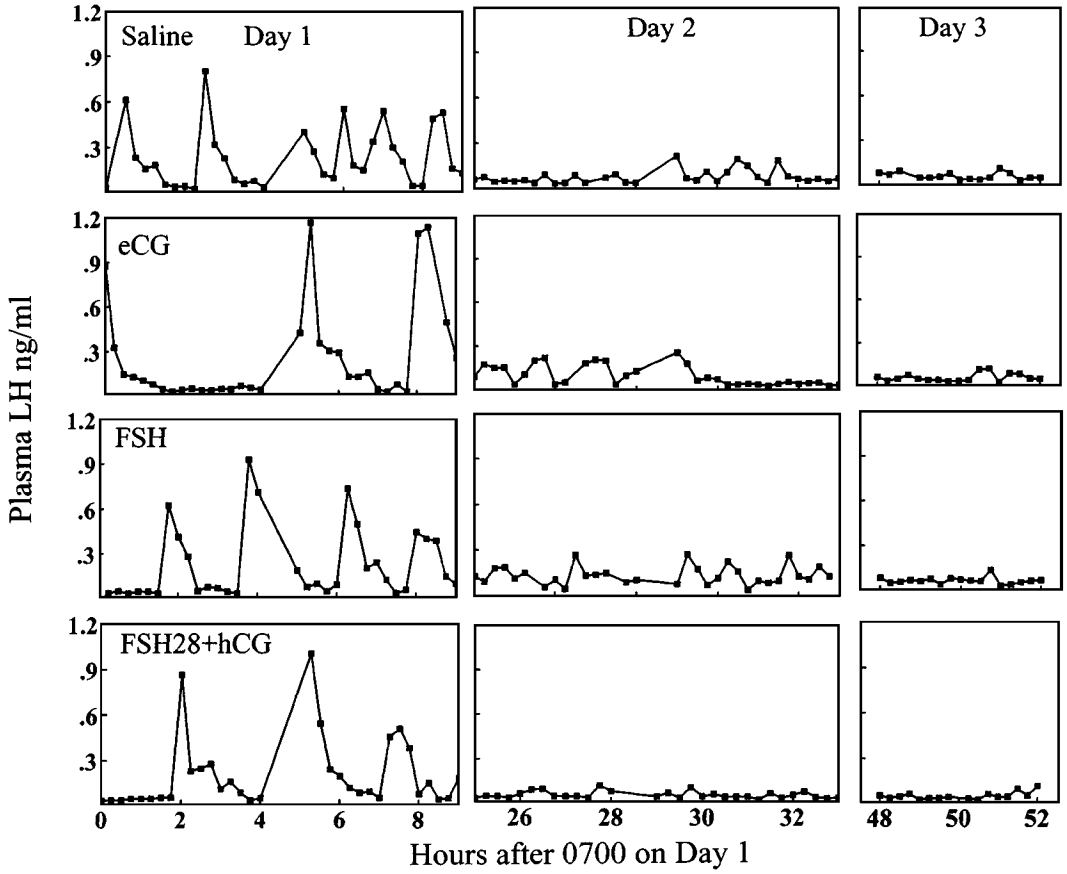

Figure 2. Luteinizing hormone (LH) concentrations in blood collected at 15-min intervals from the same gilts depicted in Figure 1.

injection (Dziuk et al., 1964), at 2400 on d 6. The cause of so many follicles failing to ovulate while other follicles in the same animals ovulate is unknown. We expected that eCG would ovulate all follicles induced to grow by FSH. Normally during the follicular phase of the estrous cycle, large follicles at various stages of development respond by ovulating after a single injection of eCG administered 1 to 4 d before the preovulatory LH surge (Hunter et al., 1976). Immature, small follicles would not have had time to grow to large size by d 7 assuming a follicle growth rate of 1 mm/d (Dailey et al., 1976; Guthrie et al., 1993).

The high numbers of large, presumptive ovulatory follicles present on d 7, even after an injection of hCG, showed that follicle development was impaired in gilts treated with the highest dose of FSH, and the sequence of events responsible for estrus and ovulation was disrupted. Secretion of estradiol increased between d 1 and 4 in all treatment groups; however, the relatively small increase in plasma estradiol in FSH28-treated gilts was not consistent with the high number of large follicles in these gilts. Plasma estradiol concentrations on d 4 did not differ among saline-, FSH14/21-, and FSH28-treated gilts. Given the number of large follicles per gilt on d 4, we conclude that estradiol secretion per follicle was less in the gilts treated with FSH28 than in saline- or eCG-treated pigs. The addition of small amounts of hCG to FSH resulted in an increase in plasma estradiol that was similar to that of eCG-treated gilts. However, the incidence of estrus was not improved; estrus was detected in only about 50% of FSH-treated gilts, compared to 100% of saline-injected gilts.

The maintenance of high plasma FSH concentrations did not affect circulating amounts of LH. Plasma LH did not differ among the treatment groups (saline, eCG, FSH28, and FSH28+hCG), showing a pulsatile secretion pattern on d 1 consistent with the proposed role of LH in the recruitment and selection of ovulatory follicles (Britt et al., 1985; Esbenshade et al., 1990; Varley and Foxcroft, 1990).

The mechanisms responsible for the detrimental effect of high plasma FSH during the follicular phase on estradiol production, estrus, and ovulation are unknown. Biological effects of FSH are expressed through activation of its receptor on the plasma membrane of granulosa cells (Ainsworth et al., 1990; LaBarbera, 1994). These receptors are coupled to adenylate cyclase so that exposure of granulosa cells to FSH leads to increased cAMP production, protein phosphorylation, and signal transduction. When FSH was added to porcine granulosa cells in culture, FSH

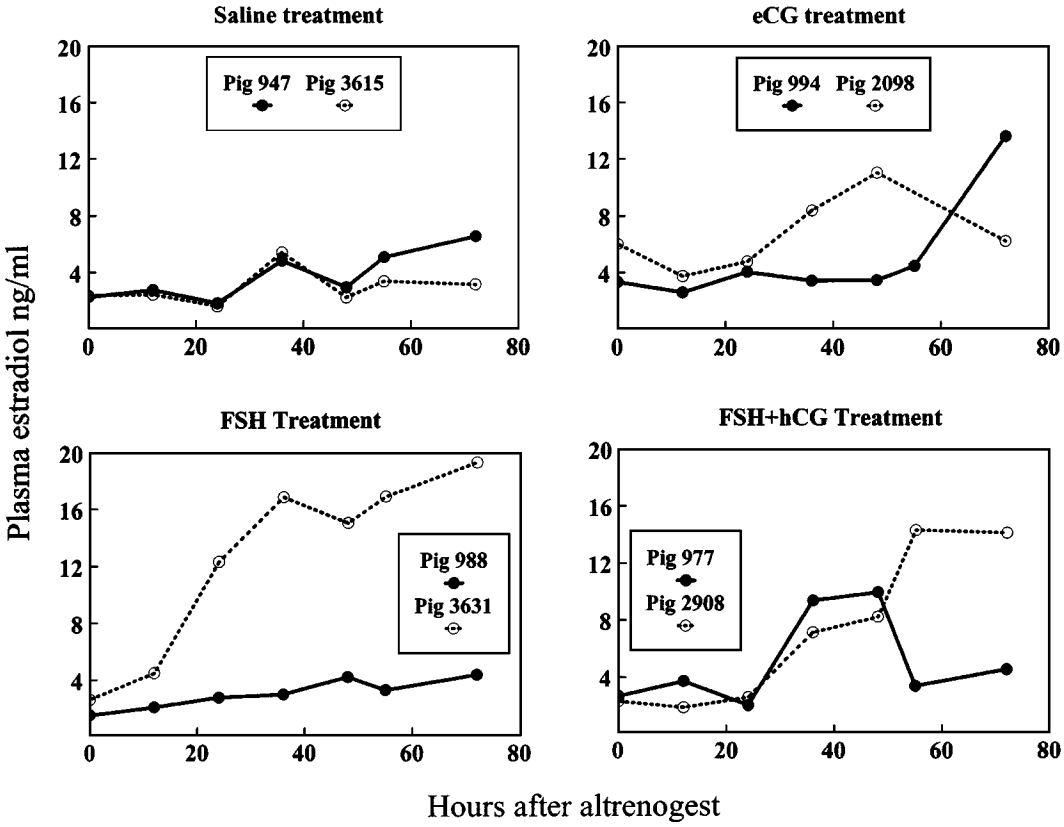

Figure 3. Estradiol concentrations in blood collected on d 1 through 4 after the last feeding of altrenogest in eight gilts. Gilts received the following treatments starting on d 1: one injection of saline at 2100, one injection of 1,200 IU of equine chorionic gonadotropin (eCG) at 2100, six injections of follicle-stimulating hormone (FSH) (total dose 28 NIH-S1 units/100 kg BW) at 12-h intervals starting at 0700 on d 1, and six injections of FSH (total dose 28 NIH-S1 units/100 kg BW) and 100 IU of human chorionic gonadotropin (hCG) at 12-h intervals starting at 0700 on d 1.

receptor site number per cell decreased (Sites et al., 1994). If FSH treatment reduces its own granulosa cell receptor, then estradiol production could be compromised because aromatase activity is dependent on FSH action in porcine granulosa cells (Ainsworth et al., 1990). However, if down-regulation of the FSH-receptor occurred, it did not interfere with FSH-induced follicular growth in this and in our earlier studies (Guthrie et al., 1988, 1990).

The conception rate (proportion of gilts with fertilized ova) was good (79 to 100%) in gilts treated with eCG, FSH14/21, or FSH28 and comparable to data for control and eCG-treated gilts in previous studies (Hunter, 1964; Webel, 1978). In contrast, co-injection of small amounts of hCG along with FSH had two detrimental effects on fertility compared to FSH28 alone; the mean percentage recovery of oocytes per gilt, based on corpora lutea number, was reduced, and the proportion of gilts with unfertilized ova was increased. A potential problem with administration of exogenous gonadotropins is the potential that they have to disrupt reproductive function outside of the ovary because their receptors are expressed in the uterus (Lei et al., 1992; Ziecik et al., 1992), oviduct (Lei et al., 1993b; Zheng et al., 1996), thyroid (Frazier et al., 1990), and brain (Lei et al., 1993a).

In conclusion, the 3-d high dose FSH treatment (FSH28) during altrenogest-synchronized follicular phase increased the number of potentially ovulatory follicles, but this potential benefit was not realized because of reduced incidence of estrus and failure of many follicles to ovulate. To use FSH to increase or induce preovulatory follicular growth in pigs, additional work is required to determine the optimum method of delivery, dose, and duration of treatment.

Implications

Although the 3-d high dose follicle-stimulating hormone (FSH) treatment protocol (FSH28) stimulated follicular growth, aspects of follicular development were abnormal because many follicles did not ovulate and the incidence of estrus was low. Co-administration of hCG with the high dose of FSH (FSH28+hCG) increased estradiol secretion and ovulation rate compared with FSH28 alone but had a negative effect on ova recovery and fertilization rate.

Additional research is required to determine the optimum method of delivery, dose, and duration of treatment before FSH can be used to increase the number of fertilizable ova in swine.

Literature Cited

Ainsworth, L., B. K. Tsang, B. R. Downey, and G. J. Marcus. 1990. The synthesis and actions of steroids and prostaglandins during follicular maturation in the pig. J. Reprod. Fertil., Suppl. 40:137–150.

Ausa. 1994. SUPER OVA®. Tech. Bull. Ausa International, Tyler, TX.

Britt, J. H., J. D. Armstrong, N. M. Cox, and K. Esbenshade. 1985. Control of follicular development during and after lactation in the sow. J. Reprod. Fertil., Suppl. 33:37–54.

Dailey, R. A., J. R. Clark, R. B. Staigmiller, N. L. First, A. B. Chapman, and L. E. Casida. 1976. Growth of new follicles following electrocautery in four genetic groups of swine. J. Anim. Sci. 43:175–183.

Day, B. N., D. E. Longenecker, S. C. Jaffe, E. W. Gibson, and J. F. Lasley. 1967. Fertility in swine following superovulation. J. Anim. Sci. 26:777–780.

Donaldson, L. E., and D. N. Ward. 1986. Some effects of LH on embryo production in superovulated cows. Vet. Rec. 119: 625–626.

Dziuk, P. J., C. Polge, and L.E.A. Rowson. 1964. Intra-uterine migration and mixing of embryos in swine following egg transfer. J. Anim. Sci. 23:37–42.

Esbenshade, K. L., A. J. Ziecik, and J. H. Britt. 1990. Regulation and action of gonadotropins in pigs. J. Reprod. Fertil., Suppl. 40: 19–32.

Frazier, A. L., L. S. Robbins, P. J. Stork, R. Sprengel, D. L. Segaloff, and R. D. Cone. 1990. Isolation of TSH and LH/CG receptor cDNAs from human thyroid: Regulation by tissue specific splicing. Mol. Endocrinol. 4:1264–1276.

Guthrie, H. D. 1977. Induction of ovulation and fertility in prepuberal gilts. J. Anim. Sci. 45:1360–1367.

Guthrie, H. D. 1979. Fertility after estrous cycle control using gonadotropin and prostaglandin $F_2\alpha$ treatment of sows. J. Anim. Sci. 49:158–162.

Guthrie H. D., and D. J. Bolt. 1985. Pituitary and ovarian hormone secretion and ovulation in gilts injected with gonadotropins during and after oral administration of progesterone agonist (Altrenogest). Biol. Reprod. 33:679–689.

Guthrie, H. D., D. J. Bolt, and B. S. Cooper. 1990. Effects of gonadotropin treatment on ovarian follicle growth and granulosal cell aromatase activity in prepuberal gilts. J. Anim. Sci. 68:3719–3726.

Guthrie, H. D., D. J. Bolt, and B. S. Cooper. 1993. Changes in follicular estradiol-17β, progesterone and inhibin immunoactivity in healthy and atretic follicles during preovulatory maturation in the pig. Domest. Anim. Endocrinol. 10:127–140.

Guthrie, H. D., D. J. Bolt, G. H. Kiracofe, and K. F. Miller. 1988. Ovarian response to injections of charcoal-extracted porcine follicular fluid and porcine follicle-stimulating hormone in gilts fed a progesterone agonist (altrenogest). Biol. Reprod. 38: 750–755.

Guthrie, H. D., D. M. Henricks, and D. L. Handlin. 1974. Plasma hormone levels and fertility in pigs induced to superovulate with PMSG. J. Reprod. Fertil. 41:361–370.

Guthrie, H. D., R. M. Rohan, and C. E. Rexroad, Jr., and B. S. Cooper. 1992. Changes in concentrations of follicular inhibin α and β_A subunit messenger ribonucleic acids and inhibin immunoactivity during preovulatory maturation in the pig. Biol. Reprod. 47:1018–1025.

Guthrie, H. D., G. R. Welch, B. S. Cooper, A. D. Zakaria, and L. A. Johnson. 1994. Flow cytometric determination of degraded deoxyribonucleic acid in granulosa cells to identify atretic follicles during preovulatory maturation in the pig. Biol. Reprod. 50:1303–1311.

Herrler, A., J. F. Beckers, J. Vivier-Donnay, and H. Niemann. 1988. Purified FSH supplemented with defined amounts of LH for superovulation in cattle. Theriogenology 29:260 (Abstr.).

Hunter, R.H.F. 1964. Superovulation and fertility in the pig. Anim. Prod. 6:189–194.

Hunter, R.H.F., B. Cook, and T. G. Baker. 1976. Dissociation of response to injected gonadotropin between the Graafian follicle and oocyte in pigs. Nature (Lond.) 260:156–158.

Koenig, J.L.F. 1987. Cytogenetic analysis of swine ova: The effect of superovulation and selection for high ovulation rate. Ph.D. Thesis. Univ. of Nebraska, Lincoln.

LaBarbera, A. R. 1994. Follicle-stimulating hormone (FSH) receptors and FSH-responsive 3′,5′-cyclic monophosphate production in porcine granulosa cells decline with follicular growth. Endocr. Res. 20:65–77.

Lei, Z. M., C. V. Rao, J. L. Kornyei, P. Licht, and E. S. Hiatt. 1993a. Novel expression of human chorionic gonadotropin/luteinizing hormone receptor gene in brain. Endocrinology 132:2262–2270.

Lei, Z. M., E. Reshef, and C. V. Rao. 1992. The expression of human chorionic gonadotropin/luteinizing hormone receptors in human endometrial and myometrial blood vessels. Endocrinology 75: 651–659.

Lei, Z. M., P. Toth, C. V. Rao, and D. Pridham. 1993b. Novel coexpression of human chorionic gonadotropin (hCG)/human luteinizing hormone receptors and their ligand hCG in human fallopian tubes. J. Clin. Endocrinol. Metab. 77:863–872.

Longenecker, D. E., and B. N. Day. 1968. Fertility level of sows superovulated at post-weaning estrus. J. Anim. Sci. 27: 709–711.

Pursel, V. G., and R. J. Wall. 1996. Effects of transferred ova per recipient and dual use of donors as recipients on production of transgenic swine. Theriogenology 46:201–209.

SAS. 1989. SAS/STAT® User's Guide (Version 6 Ed.). SAS Inst. Inc., Cary, NC.

Sites, C. K., K. Patterson, C. S. Jamison, S.J.F. Degen, and A. R. LaBarbera. 1994. Follicle-stimulating hormone (FSH) increases FSH receptor messenger ribonucleic acid while decreasing FSH binding in cultured porcine granulosa cells. Endocrinology 134:411–417.

Varley, M. A., and G. R. Foxcroft. 1990. Endocrinology of the lactating and weaned sow. J. Reprod. Fertil., Suppl. 40:47–61.

Webel, S. K. 1978. Ovulation control in the pig. In: D. B. Crighton, N. B. Haynes, G. R. Foxcroft, and G. E. Lamming (Ed.) Control of Ovulation. pp 421–434. Butterworths, London.

Zheng, W., M. S. Magid, E. E. Kramer, and Y.-T. Chen. 1996. Follicle-stimulating hormone receptor is expressed in human ovarian surface epithelium and fallopian tube. Am. J. Pathol. 148:47–53.

Ziecik, A. J., M. Jedilnska, and S. J. Rzucidllo. 1992. Effect of estradiol and progesterone on myometrial LH/hCG receptors in pigs. Acta Endocrinol. 127:185–188.